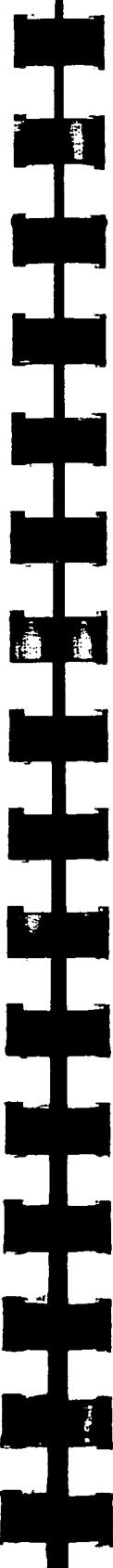


EXHIBIT 39

**Nonisotopic Probing,
Blotting, and Sequencing**

SECOND EDITION



Nonisotopic Probing, Blotting, and Sequencing

SECOND EDITION

Edited by

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Academic Press

San Diego New York Boston London Sydney Tokyo Toronto

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Front cover photograph: Color enhanced digitized image of a DNA sequence obtained using the chemiluminescent substrate CSPD to visualize bound alkaline phosphatase conjugate. This illustration was kindly provided by Irena Bronstein and Chris Martin of Tropix, Inc.

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Academic Press, Inc.
A Division of Harcourt Brace & Company
525 B Street, Suite 1900, San Diego, California 92101-4495

United Kingdom Edition published by
Academic Press Limited
24-28 Oval Road, London NW1 7DX

Library of Congress Cataloging-in-Publication Data

Nonisotopic probing, blotting, and sequencing / edited by Larry J.
Kricka

p. cm.

Includes index.

ISBN 0-12-426291-0 (hardcover) 0-12-426292-9 (comb bound)

1. Molecular probes. 2. Immunoblotting. 3. Chemiluminescence assay. 4. Bioluminescence assay. 5. Amino acid sequence. 6. Nucleotide sequence. I. Kricka, Larry J., date.

QP519.9.M65N66 1995

574.8'028--dc20

94-24916

CIP

PRINTED IN THE UNITED STATES OF AMERICA
95 96 97 98 99 00 BC 9 8 7 6 5 4 3 2 1

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Detection of Horseradish Peroxidase by Colorimetry

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1. AEC Detection
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I. INTRODUCTION

A. Historical Overview

Horseradish peroxidase (HRP) has been used extensively as a colorimetric marker in biological studies. A hemoprotein with a molecular weight of 40,000, HRP is an ideal detection reagent because of its stability, high turnover rate, and the availability of a wide assortment of colorimetric

2. Sandwich Hybridizations

Sandwich assays are particularly well suited to peroxidase detection. These assays generally include a capture probe that is bound to a fixed matrix, such as nitrocellulose membranes (Dunn and Hassell, 1977; Ranki *et al.*, 1983), microtiter wells (Dahlen *et al.*, 1987; Keller *et al.*, 1989), or beads (Langdale and Malcolm, 1985; Polsky-Cynkin *et al.*, 1985). The target nucleic acid molecule hybridizes to the capture probe and is thereby bound to the matrix, while a second probe that is either directly or indirectly labeled with peroxidase hybridizes to an adjacent sequence on the target (Fig. 2). These techniques are particularly useful for the detection of polymerase chain reaction (PCR)-amplified nucleic acids, and have been used in assays for human immunodeficiency virus (HIV) (Kemp *et al.*, 1990; Keller *et al.*, 1989), β -thalassemia (Saiki *et al.*, 1988, 1989), and sickle-cell anemia (Saiki *et al.*, 1988) among others. Peroxidase detection is useful for these types of assays because of the high turnover rate of the enzyme, and because of the availability of a number of sensitive substrates for soluble assays.

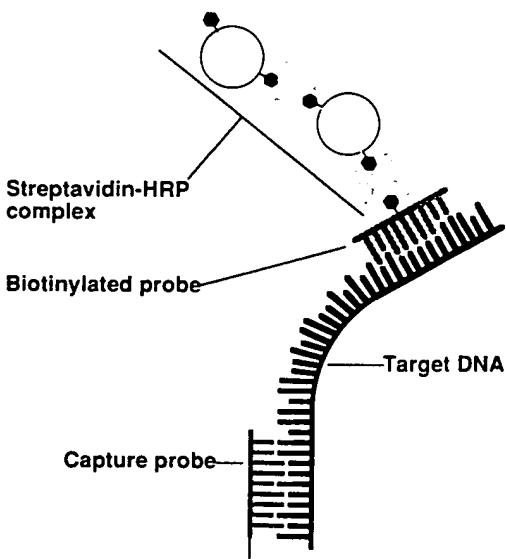


Fig. 2—Sandwich assays. The target hybridizes to an immobilized probe and is captured to the solid matrix. A biotinylated probe hybridizes to adjacent sequences on the target and is detected by streptavidin-HRP.

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